Nephrol Dial Transplant (2008) 23: 734–740
doi: 10.1093/ndt/gfm761
Advance Access publication 5 January 2008

Short Communication

NDT
Nephrology Dialysis Transplantation

Elevated asymmetric dimethylarginine (ADMA) and inverse correlation between circulating ADMA and glomerular filtration rate in children with sporadic focal segmental glomerulosclerosis (FSGS)

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Abstract

Background. Steroid-resistant nephrotic syndromes (NS) with focal and segmental glomerulosclerosis (FSGS) can be differentiated into sporadic and syndromic forms. In sporadic NS, a circulating FSGS-factor is discussed in the pathogenesis and is thought to inhibit the synthesis of nitric oxide (NO) from L-arginine by blocking the NO synthase (NOS). Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of all types of NOS. In a previous study we did not find an elevation of ADMA in a syndromic form of FSGS, the Schimke-immuno-osseous dysplasia. Here we report for the first time data on the L-arginine/NO pathway in sporadic FSGS of childhood.

Methods. Nine children (5 to 18 years of age) suffering from sporadic FSGS and age-matched healthy controls were investigated. ADMA in plasma and urine as well as L-arginine in plasma were determined by gas chromatography–tandem mass spectrometry. The NO metabolites nitrate and nitrite were measured in plasma and urine by gas chromatography–mass spectrometry (GC-MS). The ADMA metabolite dimethylamine (DMA) was measured in urine by GC-MS.

Results. We found elevated plasma levels of ADMA in children suffering from sporadic FSGS compared to healthy controls (851 nmol/L versus 684 nmol/L, $P = 0.008$). An inverse correlation between ADMA and glomerular filtration rate (GFR) was found in sporadic FSGS (Pearson's correlation coefficient -0.784, $P = 0.012$).

Conclusion. Our study suggests that ADMA synthesis is elevated in sporadic FSGS. This finding argues for the involvement of ADMA in the pathogenesis of this disease in childhood.

Keywords: ADMA; DMA; FSGS; NO pathway; sporadic

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Introduction

Focal and segmental glomerulosclerosis (FSGS) represent a histological variant of nephrotic syndromes (NS) in childhood [1]. There exist sporadic and syndromic forms of FSGS. Syndromic forms like the Schimke-immuno-osseous dysplasia (SIOD) are a subvariant of genetic forms of FSGS, usually showing no recurrence of FSGS in transplanted kidneys [2].

In sporadic forms of FSGS, the disease recurs in 30–40% of transplanted patients [1]. In contrast to syndromic forms of FSGS, an intrinsic renal disease is unlikely to be the pathogenetic cause in sporadic FSGS. There is evidence for a circulating factor playing a role in the pathogenesis and recurrence of FSGS, by modifying the glomerular permeability to albumin [3]. It has been speculated that this so-called FSGS-factor inhibits the synthesis of nitric oxide (NO) from L-arginine by blocking NO synthase (NOS), thereby antagonizing the antifibrotic effect of NO within the mesangium resulting in progressive glomerulosclerosis [4].

The NOS are a family of enzymes that convert L-arginine to L-citrulline and NO. The activity of NOS is effectively controlled by the endogenous inhibitor asymmetric dimethylarginine (ADMA) [5]. ADMA is produced by methylation of protein-associated L-arginine via *N*-methyl protein transferases and subsequent regular proteolysis [6].

NO is a gaseous, freely diffusible molecule with multiple physiological functions including vasodilation, inhibition of platelet aggregation and adhesion [7]. NO is involved in the regulation of regional blood flow and systemic blood pressure. Inhibition of NOS by ADMA induces vascular dysfunction in man [8]. Additionally, suggesting a causal relationship between NO-mediated vasodilation and atherosclerosis, studies in the cholesterol-fed rabbit model for atherosclerosis showed that administration of L-arginine prevented the loss of endothelial-dependent vasodilation [9]. In that study, hypercholesterolaemic rabbits were found to have elevated plasma concentrations of ADMA [9].

Table 1. Parameters of L-arginine/NO pathway in healthy controls, patients with FSGS and non-FSGS renal diseases

	Aged-matched healthy controls	FSGS	Non-FSGS renal diseases
ADMA plasma (nmol/L) mean (SD)	683 (106)	851 (108)*	788 (130)
Arginine plasma (μmol/L) mean (SD)	77 (29)	56 (14)	60 (11)
Nitrate plasma (μmo/L) mean (SD)	91 (73) $n = 7$	71 (24)	115 (60)
Nitrite plasma (μmol/L) mean (SD)	3.2 (1.1) $n = 7$	2 (1.1)	3 (1.5)
ADMA urine (μmol/mmol creatinine) mean (SD)	15.7 (26)	41 (55)	5.7 (6)
DMA urine (μmol/mmol creatinine) mean (SD)	160 (248)	345 (489)	130 (155)
Nitrate urine (μmol/mmol creatinine) mean (SD)	421 (712)	262 (419)	174 (204)
Nitrite urine (μmol/mmol creatinine) mean (SD)	1.3 (2)	3.0 (5)	1.3 (1.5)
Age (years) mean (SD)	11 (4.6)	11 (4.2)	4 (2.8)
Number of children	9	9	11
GFR (ml/min/1.73 m^2bs) mean (SD)	nd	72 (55)	32 (44) $n = 9$
Hypertension	no	$n = 5$	$n = 5$
Cholesterol (mg/dL)	nd	269	212
Serum protein (g/L)	nd	65 (11)	60 (20)
Protein/creatinine in urine (g/mmol)	nd	0.3 (0.5)	1.4 (1.9) $n = 8$

*$P < 0.05$ versus controls (GFR = glomerular filtration rate, m^2bs = square metre body surface, nd = not determined).

Elevated ADMA levels have been measured in plasma of adults with decreased renal function [10], but the role of the kidney in accumulation of ADMA is contradictory. It is believed that the kidney plays a role in the direct elimination of ADMA [11]. However, the greatest fraction (∼80%) of ADMA is assumed to be first hydrolysed by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) to L-citrulline and dimethylamine (DMA) [6]. DDAH is highly expressed in the liver and in the kidney [12]. In humans, systemic infusion of ADMA in healthy adults caused a dose-dependent decrease of renal plasma flow, with glomerular filtration rate (GFR) remaining unchanged, a decrease in cardiac output and an increase of systemic vascular resistance and blood pressure [13]. Since elevated circulating levels of ADMA have been found in a variety of diseases, most of them being associated with NO-dependent endothelial dysfunction [14,15], ADMA is considered a novel marker for cardiovascular risk in adults [8,10].

Recently we reported that the L-arginine/NO pathway is not involved in the pathogenesis of SIOD, a syndromic form of FSGS [16]. The aim of the present study was to assess the L-arginine/NO pathway in children with sporadic FSGS and to investigate a potential role of ADMA in this disease.

Subjects and methods

Nine children with sporadic FSGS and age-matched healthy controls were investigated. These groups were compared to 11 children with renal diseases other than FSGS (non-FSGS) as pathological controls. The characteristics of the patients included in the present study are summarized in Table 1 (sporadic FSGS) and Table 2 (non-FSGS). The patients were not tested for FSGS mutations. The study was approved by the Ethics Committee of Hannover Medical School and written informed consent was obtained from the parents.

The synthesis and elimination of ADMA were assessed by measuring circulating and excretory ADMA as well as excretory DMA. ADMA in plasma and urine as well as L-arginine in plasma were determined by gas chromatography–tandem mass spectrometry (GC-MS-MS) as described elsewhere [17]. Urinary DMA was measured by gas chromatography–mass spectrometry (GC-MS) [18].

NO synthesis was assessed by measuring the plasma and urinary concentrations of nitrite and nitrate, the major NO metabolites and indicators of NO synthesis [19]. Nitrate and nitrite in plasma and urine were determined simultaneously by GC-MS, as described previously [16,20].

Urinary excretion of all biochemical parameters was corrected for creatinine excretion. Creatinine concentration in urine was determined by high-performance liquid chromatography (HPLC) [16,21].

Sample size varied due to limited urine and blood volumes available in young children. GFR was calculated using the Schwartz formula.

Data from patients and healthy controls were compared using the Wilcoxon test (SPSS, version 13). The non-FSGS group was compared with controls or the FSGS group using the Student's *t*-test for unpaired samples. Values of $P < 0.05$ were considered significant. Data were presented as mean ± SD.

Results

Plasma levels of ADMA in nine patients with sporadic FSGS were significantly higher than in healthy controls (851 ± 108 versus 683 ± 106 nmol/L, $P = 0.008$), though there was some overlap (Figure 1A). In the sporadic FSGS group, children with hypertension had insignificantly ($P = 0.13$) higher ADMA plasma levels as compared to normotensive children (896 ± 48 versus 788 ± 130 nmol/L). Plasma levels of ADMA were higher in the group of children with sporadic FSGS than in children with renal diseases other than FSGS (non-FSGS); however, this difference was not statistically significant ($P = 0.44$). Furthermore, the elevation of ADMA plasma levels in the non-FSGS group was not statistically different compared to healthy controls ($P = 0.26$). In addition, within the non-FSGS group, patients without dialysis or transplantation did not show differences in plasma ADMA levels compared to patients with dialysis or transplantation.

Table 2. Characteristics of patients with sporadic FSGS

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Age (years)	18	6	5	10	16	8	14	15	10
HD	no	yes	no	no	no	no	no	no	no
Tx	yes	no	no	no	no	no	no	no	no
GFR (mL/min/1.73 m^2bs)	4.9	nc	7.9	43.8	49.3	62.1	111.7	126.7	139.7
ADMA plasma (nmol/L)	945	892	966	933	885	749	781	655	824
Creatinine (μmol/L)	563	35	486	101	135	82	50	48	34
Cholesterol (mg/dL)	155	197	480	445	267	282	224	182	97
Hypertension	yes	yes	no	yes	yes	no	no	no	yes
Serum protein (g/L)	78	72	47	46	66	67	64	79	68
Protein/creatinine in urine (g/mmol creatinine)	0.09	0.02	0.77	1.43	0.04	0.04	0.01	0.05	0.02
Medication	CSA, sirolimus, furosemide, losartan	Metoprolol, nifedipine, prazosin	Nifedipine	CSA, prednisone, losartan	CSA, mycophenolate mofetil, prazosin, ramipril	CSA	CSA, prednisone	CSA	Prednisone
Nephrotic syndrome	Complete remission	No remission	Partial remission	Partial remission	Complete remission	Complete remission	Complete remission	Complete remission	Complete remission

HD = haemodialysis, Tx = transplantation, GFR = glomerular filtrations rate, m^2bs = square metre body surface, nc = not calculated because of HD, CSA = cyclosporine A.

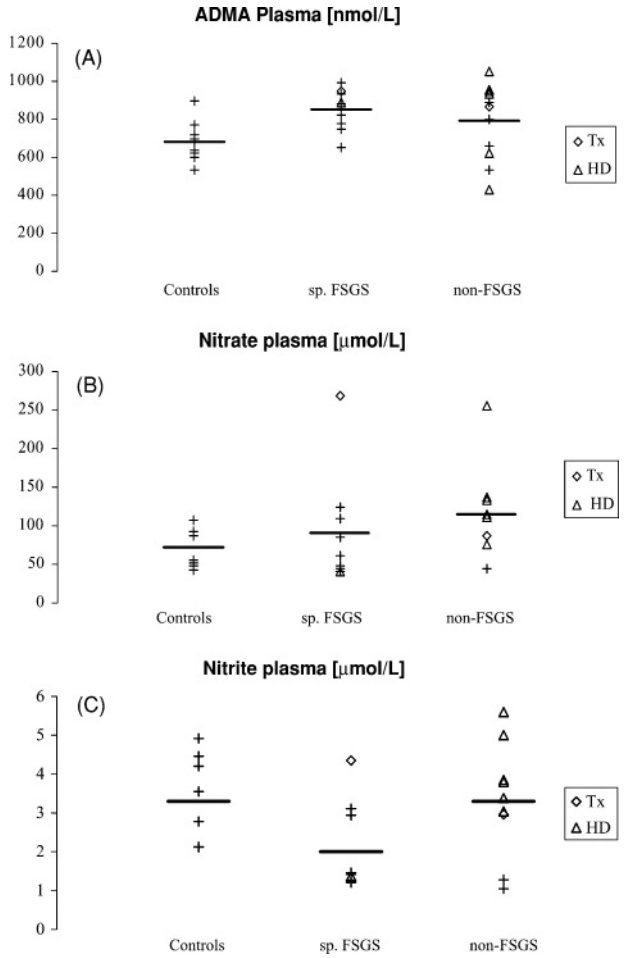

Fig. 1. Plasma concentrations of ADMA (A), nitrate (B) and nitrite (C) in healthy controls ($n = 9$) and patients with sporadic FSGS (total: $n = 9$; Tx: $n = 1$; HD: $n = 1$) and non-FSGS (total: $n = 11$; Tx: $n = 1$; HD: $n = 6$). Data from patients under dialysis and after transplantation are indicated by rhombus and triangle, respectively. sp. FSGS = sporadic FSGS.

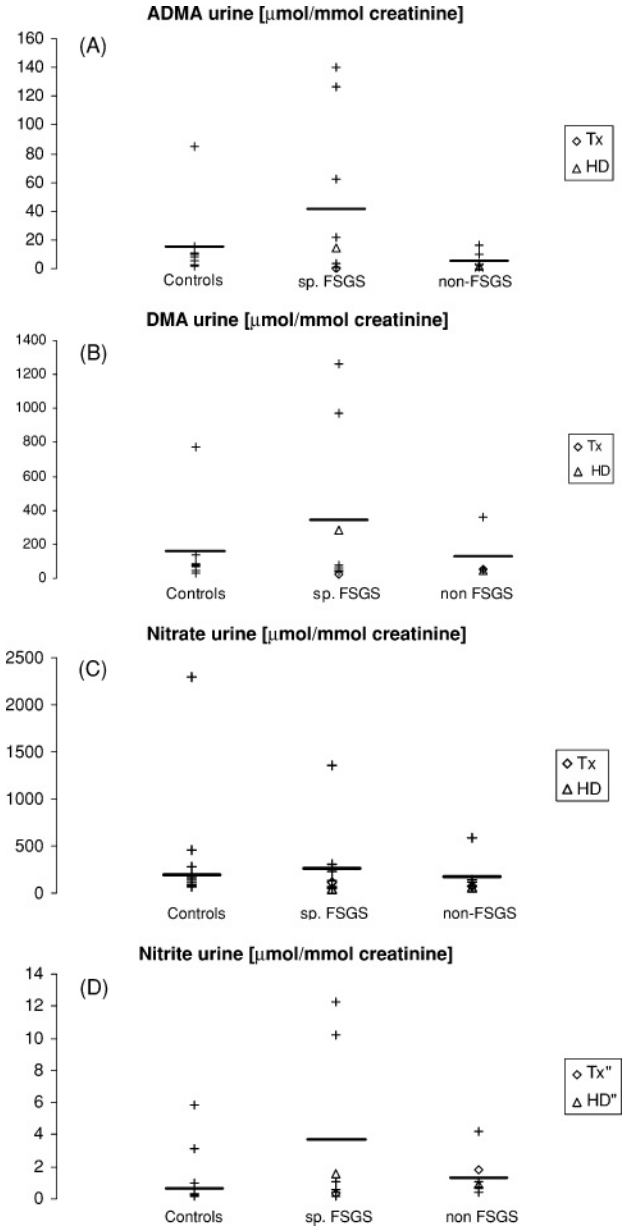

Fig. 2. Creatinine-corrected excretion rates of ADMA (A), DMA (B), nitrate (C) and nitrite (D) in healthy controls ($n = 9$) and patients with sporadic FSGS (total: $n = 9$; Tx: $n = 1$; HD: $n = 1$) and non-FSGS (total: $n = 11$; Tx: $n = 1$; HD: $n = 6$). Data from patients under dialysis and after transplantation are indicated by rhombus and triangle, respectively. sp. FSGS = sporadic FSGS.

Renal excretion of ADMA was slightly but not significantly higher in patients with sporadic FSGS (41.4 ± 55 versus 15.7 ± 26 μmol/mmol creatinine, $P = 0.314$) compared to healthy controls (Figure 2A; Table 3). Compared to healthy controls, creatinine-corrected excretion of DMA in urine was tendentiously higher in patients with sporadic FSGS (345 ± 489 versus 160 ± 248 μmol/mmol creatinine, $P = 0.06$; see Figure 2B; Table 1). DMA excretion rate in the non-FSGS group was not statistically different compared to healthy controls ($P = 0.73$).

In plasma, nitrate (91.4 ± 73 versus 71.3 ± 24 μmol/L, $P = 0.779$; Figure 1B) and nitrite (2.0 ± 1.1 versus 3.2 ± 1.1 μmol/L, $P = 0.093$) (Figure 1C) of the FSGS group were not significantly different from healthy controls. Creatinine-corrected excretion of nitrate (262 ± 419 versus 421 ± 712 μmol/mmol creatinine, $P = 0.314$; Figure 2C) and nitrite (3.0 ± 5.0 versus 1.3 ± 2 μmol/mmol creatinine, $P = 0.2$; Figure 2D) of the FSGS group did not differ statistically from healthy controls. L-Arginine plasma levels were not statistically different between the groups ($P = 0.11$, Table 3).

An inverse correlation (Pearson's correlation coefficient (r) −0.784, $P = 0.012$) was found between ADMA in plasma and GFR in non-HD (haemodialysis) sporadic FSGS patients (Figure 3), but not in non-FSGS children (not shown). No statistically significant differences were found between the GFR of the FSGS and the GFR of the non-FSGS group ($P = 0.8$; HD patients were excluded). No correlation was found between urinary DMA and ADMA ($r = 0.625$, $P = 0.097$) or between urinary DMA and GFR ($r = -0.2$, $P = 0.64$) in the sporadic FSGS group.

Table 3. Characteristics non-FSGS patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11
Diagnosis	Alport syndrome	Renal hypoplasia	Obstructive uropathy	Branchio-oto renal syndrome	Schönlein Henoch nephritis	Joubert syndrome	ARPKD	ARPKD	ARPKD	Minimal change nephritic syndrome	ARPKD
Age (years)	14	19	20	10	12	13	8	4	1	4	11
HD	yes	yes	yes	yes	yes	yes	no	no	no	no	no
Tx	no	no	no	no	no	no	no	no	no	no	yes
GFR (mL/min/1.73 m^2bs)	nc	nc	nc	nc	nc	nc	47	127.9	90.7	140	27.3
ADMA plasma (nmol/L)	930	426	622	949	1051	959	865	887	536	799	656
Creatinine (μmol/L)	623	640	1113	670	865	780	128	40	36	21	78
Cholesterol (mg/dL)	278	200	203	278	123	186	220	nd	nd	189	205
Hypertension	no	no	yes	yes	no	yes	no	yes	no	no	yes
Serum protein (g/L)	78	69	79	60	65	72	77	77	67	40	76
Protein/creatinine in urine (g/mmol)	nd	nd	nd	2.7	0.6	0.2	0.02	0.04	0.04	0.68	0.09
Medication	Nifedipine, calcitriol	Calcitriol	Calcitriol	Calcitriol	Nifedipine, metoprolol	Calcitriol	Furosemide, metoprolol	Enalapril, losartan	Furosemide, nifedipine, captopril	Furosemide, nifedipine, captopril	Cyclosporine, prednisone

HD = haemodialysis, Tx = transplantation, GFR = glomerular filtrations rate, m^2bs = square metre body surface, nd = not determined, nc = not calculated because of HD, ARPKD = autosomal recessive polycystic kidney disease.

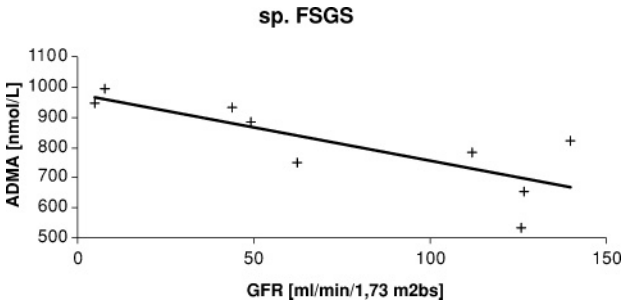

Fig. 3. Correlation between plasma ADMA concentration and GFR in sporadic FSGS (non-HD) patients. Pearson's correlation coefficient $r = -0.784$, $P = 0.012$. sp. FSGS = sporadic FSGS.

The sporadic FSGS group consisted of five children with hypertension and four normotensive patients. GFR values did not differ statistically in these subgroups. In both groups, a trend of negative correlation was found between ADMA plasma levels and GFR.

Discussion

NS is associated with an increased risk for cardiovascular disorders; thromboembolic complications and premature atherosclerosis are typical findings in adults and even in children [1]. However, data on the status of the L-arginine/NO pathway in nephrotic children are rare. Recently, we showed that this pathway does not play a role in the pathogenesis of SIOD, a syndromic form of FSGS [16]. Importantly, plasma levels of ADMA in 10 SIOD patients (691 ± 168 nmol/L) were not significantly different ($P = 0.78$) from those in age-matched controls (677 ± 138 nmol/L) [16].

To our knowledge, the present study is the first to investigate the L-arginine/NO pathway in children with sporadic FSGS. We found that these children had elevated levels of ADMA in plasma compared to healthy controls. In our study, GFR of the sporadic FSGS group and the non-FSGS group did not show significant differences, while only in the sporadic FSGS group was ADMA in plasma significantly elevated compared to healthy controls. Thus, impairment of renal function does not explain our finding of elevated ADMA levels in plasma of children with sporadic FSGS. Urinary excretion rates of ADMA and DMA in patients with sporadic FSGS were even higher than those of healthy controls, also suggesting that the elevation of ADMA levels in the circulation did not result from an impaired renal elimination of ADMA or a reduced activity of DDAH in sporadic FSGS.

Cholesterol levels were slightly higher in the group of patients with sporadic FSGS compared to the group of patients with non-FSGS (Table 3), which was to be expected if the non-FSGS renal diseases were not nephritic. However, we do not think that the slight elevation of cholesterol can explain the elevated ADMA in plasma of children with FSGS, because even in children with hypercholesterolaemia type IIa, circulating ADMA levels did not differ significantly compared to age-matched healthy controls [22].

The L-arginine/NO pathway may influence the differentiation and mobilization of endothelial progenitor cells (EPcC) [23], which replace damaged endothelial cells in vaso-occlusive vessels [24]. EPC were found to be reduced in vaso-occlusive diseases, as in coronary artery disease [25]. ADMA has been shown to suppress EPC in coronary artery disease [26]. Taking into account that sporadic FSGS can be regarded as a renal vaso-occlusive disease, elevated ADMA levels might decrease EPC in this disease, thereby causing a progression of renal vascular lesions and decreasing the GFR. The findings of the present study, of elevated plasma concentration of ADMA in sporadic FSGS, and the inverse relationship between ADMA in plasma and GFR in patients with sporadic FSGS, but not in children with other renal diseases, are supportive of this hypothesis.

A negative relationship between ADMA plasma concentration and GFR has been reported for hypertensive children and adolescents [27]. The subgroups of hypertensive and normotensive patients of the sporadic FSGS group of the present study are rather small and do not allow reliable comparison. Nevertheless, our findings suggest that the inverse correlation of circulating ADMA levels and GFR found in the sporadic FSGS group of our study is likely to be associated with the renal disease rather than with hypertension. In healthy adults, short-term infusion of ADMA has been shown not to affect GFR at all [13]. Presumably, this apparent discrepancy may be explained by the duration of action of ADMA, which is rather chronic in sporadic FSGS patients.

Since patients with sporadic FSGS show a high percentage of recurrence of the disease after transplantation, ADMA concentrations in plasma may serve as a follow-up parameter for the severity of sporadic FSGS or recurrence of the disease after renal transplantation. However, due to small sample size, subgroup analysis is limited in our study. Ongoing studies of our group focus on the influence of dialysis and kidney transplantation on ADMA synthesis and elimination in childhood.

Recently, we presented an anthropometric instrument to distinguish SIOD, a syndromic form of FSGS, from non-SIOD renal diseases [28]. ADMA levels in plasma of patients with this syndromic form of FSGS were normal [16]. Since, we have now found elevated plasma levels of ADMA in children with sporadic FSGS, the measurement of ADMA in plasma in combination with anthropometric parameters may be an appropriate tool to distinguish syndromic from sporadic forms of FSGS. However, further studies are necessary to prove this hypothesis.

In summary, elevated ADMA concentrations in plasma of children suffering from sporadic FSGS, presumably resulting from enhanced ADMA synthesis, suggest a role of ADMA in the pathophysiology of this nephrotic syndrome.

Abbreviations

ADMA, asymmetric dimethylarginine; DMA, dimethylamine; EPC, eudothelial progenitor cells; FSGS, focal segmental glomerulosclerosis; GFR, glomerular filtration rate; NO, nitric oxide; NOS, NO synthase; non-FSGS,

renal disease other than FSGS; SIOD, Schimke-immuno-osseous dysplasia

Acknowledgements. We would like to thank B. Beckmann and A. Mitschke for excellent laboratory assistance and F.-M. Gutzki for performing GC-MS and GC-MS-MS analyses. We are grateful to Dr B. Vaske, Biometric Institute, Medical School of Hannover, Hannover, Germany, for helping us with statistical analysis. K.C. is recipient of a grant (A/06/08992) from the DAAD (Deutscher Akademischer Austauschdienst).

Conflict of interest statement. The authors have no conflict of interest to declare.

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Received for publication: 19.10.06
Accepted in revised form: 24.9.07